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EXAMINER	
SWITZER, JULIET CAROLINE	
ART UNIT	PAPER NUMBER
1634	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/856,333	BERLIN, KATHRIN	
	Examiner	Art Unit	
	Juliet C. Switzer	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11 September 2006*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-9,11-16,18-24,26,30 and 31* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9,1-9, 11-16, 18-24, 26, 30 and 31* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *9/11/06*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. This action is written in response to applicant's correspondence submitted 9/11/06. Claims 1, 26, and 30 was amended, claim 27 was canceled, and claim 31 was added. Claims 1-9, 11-16, 18-24, 26, 30 and 31 are pending. Applicant's amendments and arguments have been thoroughly reviewed, but are not persuasive for the reasons that follow. Any rejections not reiterated in this action have been withdrawn. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action. **This action is FINAL.**

Claim Rejections - 35 USC § 103

2. Claims 1-6, 8, 18-24 and 31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rice *et al.* (Oncogene (1998) 17, 1807-1812) in view of Gifford (US 5750335).

Rice *et al.* teach a method for identifying 5-methylcytosine positions in a sample genomic DNA, said method comprising the steps of:

(a) chemically treating a sample genomic DNA obtained from at least one cell in such a way that cytosine and 5-methylcytosine react differently and from products with different base pairing behavior (p. 1811, treatment with sodium bisulfite);

(b) amplifying by means of a polymerase reaction a segment of the genomic DNA obtained in step (a) (p. 1811, second column);

(c) performing steps (a) and (b) on a reference genomic DNA (Figure 3, methylation was determined for eight different cell types, including normal lymphocytes and normal mammary epithelial cells);

Rice *et al.* utilize chemical treatment with sodium bisulfite in a method to identify the location of methylated cytosines in genomic DNA. Treatment with bisulfite results in the conversion of unmethylated cytosine residues to uracil, while methylated cytosine residues remain unchanged. Thus, in a sample where every cytosine was methylated there would be no change in sequence, but in a sample with very low levels of methylation, after PCR there would be thymines where the unmethylated cytosines previously were located. Rice *et al.* effectively introduce mutations nucleic acid sequences via the treatment with sodium bisulfite. When two treated samples are compared, for example a sample that was highly methylated versus one that had low levels of methylation, the treated sequences would have sequence differences at each position where there was differential methylation. Rice *et al.* compare the amplified sequences to determine the methylation positions after amplification of the sequences, comparing the sequences to the known BRAC1 sequence (p. 1811, 2nd column).

With regard to claim 2, in the method taught by Rice *et al.* positions which are variable between different cell lines are identified (see figure 3).

With regard to claim 3, Rice *et al.* utilize a bisulfite to treat the genomic DNA.

With regard to claim 4, Rice *et al.* jointly amplify genomic DNA from several cells, as they necessarily isolated DNA from more than one cell for each cell line.

With regard to claim 5, Rice *et al.* separately amplified the DNA from several cell lines, and then treated them all with bisulfite.

With regard to claim 8, Rice *et al.* test unmethylated cell lines (p. 1807, second column; Figure 3).

With regard to claim 18, a nucleotide sequence is considered a "chemical function" that enables a PCR product to be immobilized on a surface. Therefore, the PCR carried out by Rice *et al.* necessarily uses a primer that enables the polymerase reaction to be immobilized on a surface. The claim does not actually require an immobilization step.

Rice *et al.* do not detect the differences between amplified sequences by forming heteroduplexes from the amplified products for the comparison of a test and reference sample, and Rice et al. do not teach a method of determining the methylated positions "without determining the entire sequence of the segment," as required by newly added claim 31.

Gifford teaches a method for identifying sequence differences between two nucleic acids that comprises the steps of:

(d) forming heteroduplexes from two different nucleic acid samples (Col. 3, lines 40-50);

(e) introducing a detectable label into the heteroduplexes of step (d) by means of a reaction which is specific for non-complementary base pairs (Col. 4, lines 15-20), and

(f) determining, without sequencing, the position of mismatches in the sample genomic DNA based on the presence and position of the detectable label (also Col. 5, lines 1-12).

In particular, Gifford teaches methods for detecting mismatches in nucleic acid samples by forming heteroduplexes and binding those heteroduplexes with a heteroduplex binding protein (abstract and throughout). Gifford provides a method for determining the position of the mismatch in a heteroduplex based identifying the presence and position of the detectable label for example by binding sample nucleic acids to reference nucleic acids that are immobilized on a solid support, binding mismatches with a mismatch binding protein and detecting the mismatch binding protein as an indication of a mismatch (Col. 5, lines 1-12). This method determines the

presence and position (i.e. that there is a mismatch within a particular nucleic reference nucleic acid) of the mismatch and of the label "without sequencing." The presence and position of the sequence difference has been determined, at least it is known that the mismatch is within hybrid that has been bound by the binding protein.

Gifford specifically teaches comparing a sample (patient) nucleic acid fragment with a control (normal) nucleic acid fragment (Figure 2).

With regard to claims 4 and 5, Gifford teaches that a test or reference nucleic acid may include monoclonal or polyclonal cell lines (Col. 9, 22-25).

With regard to claims 19, Gifford teaches that the reference or test nucleic acids may be immobilized to a solid surface (Col. 5, lines 1-5; col. 13, lines 1-5). With regard to claim 20, Gifford teaches that "different" reference nucleic acids may be immobilized on a solid surface at different spots, which are considered different reaction vessels (Col. 5, lines 1-5). Further, Gifford teaches the transfer of the amplified products to different vessels (affinity columns or affinity matrix) for purification of the heteroduplexes wherein the products are coupled to a solid support (column 5, lines 47-56).

With regard to claim 21 and 22, Gifford teaches an using an enzyme that forms a complex with a non-complementary base pair (Col. 4, lines 10-20), specifically teaching MutS (Col. 7, line 22).

With regard to claim 23, Gifford teaches a method wherein the enzyme bears a label by which a complex can be displayed (Col. 15, lines 65-67).

With regard to claim 24, Gifford teaches that the label is a fluorescence label (Col. 15, lines 65-66).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the methylation detection method taught by Rice *et al.* so as to have utilized the mutation detection methods taught by Gifford. One would have been motivated to utilize the methods taught by Gifford in order to achieve the express benefits of the methods taught by Gifford which include achieving "rapid and accurate genetic screening and diagnosis by comparing two nucleic acids for differences in their sequences... (Col. 3, lines 23-25)." The combination of the two methods would have resulted in the practice of the claimed methods, and as such, the claimed invention is prima facie obvious. With regard to claim 6, the result required in claim 6 would have been a necessary property of the practice of the assay taught by Rice *et al.* in view of Gifford. Namely, where there was differential methylation between the sample genomic DNA and any of the reference DNA's taught by Rice *et al.,* when these were subjected to heteroduplex analysis as taught by Gifford erroneous base pairings would have been produced at the positions at which 5-methylcytosine was located in the sample genomic DNA but not in the reference genomic DNA.

3. Claims 9, 11, 12, 13, 14, 15, 16, 26, 30 and 31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rice *et al.* in view of Gifford as applied to claims 1-6, 8, 18-24 and 31 above, and further in view of Koster *et al.* (US 6428955).

The teachings of Rice *et al.* in view of Gifford are applied herein as applied in the previous rejection. In the method taught by Rice *et al.* in view of Gifford, sodium bisulfite is used which results in the modification of unmethylated cytosines, and therefore mismatches would occur among treated nucleic acids at positions where there was relative differential methylation of sequences. Thus, after bisulfite treatment, one is left with nucleic acids that have

mismatches relative to one another if the sequences are differentially methylated. Rice *et al.* in view of Gifford do not teach a method in which the heteroduplex is detected by cleavage of the heteroduplex molecule or in which mass spectrometry is used to analyze the size of the DNA fragments.

Claim 26 differs from claim 1 in that in step (b) the PCR primer is fluorescently labeled and provided with a chemical function to enable the immobilization of the amplificate on the surface, step (c) sets forth immobilizing the amplificate on a surface, step (f) utilizes a chemical mismatch cleavage methodology, and step (g) utilizes mass spectrometry, whereby in step (h) the presence or presence and position of the 5-methycytosine within the genomic DNA is deduced from the length of the cleaved nucleic acids.

Likewise, claim 30 is similar to claim 26, and thus also similar to claim 1. Steps (a)-(d) of claim 30 are similar to those of claim 1. Unlike claim 1, however, claim 30 requires that the heteroduplexes of step (d) are cleaved by a chemical mismatch cleavage reaction. Claim 9 depends from claim 30 and requires that the nucleic acid backbone of the heteroduplex is specifically cleaved at the non-complementarily base paired positions by an enzymatic means. Claims 11-15 also depend from claim 30.

With regard to claims 11-14, Koster *et al.* teach methods for analyzing the size of nucleic acid fragments using mass spectrometry, specifically teaching the use of MALDI-TOF and ESI, (Col. 18, line 66-Col. 19, line 11).

With regard to claim 15, which requires that the nucleic acids in step (e) are "adapted" to the performance capacity of the mass spectrometer, Koster *et al.* teach utilizing a variety of PCR amplification methods to obtain PCR products that they analyze using the mass spec (See

examples 14-15, for example). With regard to claim 16, Koster *et al.* teach utilizing nested PCR to amplify products for detection (Examples 5 and 14, for example), a method which uses primers that are set stepwise along the DNA with respect to the inner and outer pairs of primers and produce a series of amplification products, at least one which is within the mass range detectable by means of mass spectrometry. These PCR primers are considered to be "set stepwise" since they amplify two differently sized products where on is a size "step" down from the other.

Koster *et al.* teaches a method in which a heteroduplex is cleaved by an agent that cleaves the unhybridized portion so that a mismatch results in two products and then detecting these by mass spectrometry to detect the presence of the mismatch (Col. 5, lines 30-40; Col. 23, lines 25-40). Koster *et al.* further teach primers that are labeled with biotin (a means for immobilizing an amplificate on a surface; col. 35, for example) and primers that are labeled with a radioactive label and oligonucleotides that are fluorescently labeled (Col. 49, for example). In addition, as noted previously in this office action, any nucleic acid sequence itself is considered a "chemical function" that would enable the immobilization of the amplificate on a surface. Nonetheless, Koster further teach methods in which the sequence to be detected is immobilized to a solid support by means of hybridization (Col. 3, lines 60-67) or by means of a biotin/streptavidin immobilization or other reversible or irreversible bond (Col. 16, lines 7-22).

It would have been prima facie obvious to one of ordinary skill in the art to have modified the method taught by Rice *et al.* in view of Gifford *et al.* so as to have used the amplification and detection methods taught by Koster *et al.* One would have been motivated to use mass spectrometry as a means for detection of nucleic acid fragments in order to take

advantage of the express benefits of such a method as taught by Koster *et al.*, who state "the processes of the invention provide for increased accuracy and reliability of nucleic acid detection by mass spectrometry (Col. 5, lines 62-65)." One would have been motivated to use chemical cleavage to detect heteroduplexes by Koster's specific suggestion to do so, and in order to take advantage of an additional method for detecting sequence differences among the potentially differentially methylated sequences taught by Rice et al. Furthermore, it would have been prima facie obvious to have utilized fluorescently labeled primers in place of the radioactively labeled primers taught by Koster *et al.* in order to have provided an alternative labeling method that is safer to use as opposed to using radioactivity in the laboratory. Regarding claim 31, the combined methods would have clearly provided an additional method for identifying the position of the methylated positions without sequencing the entire sequence of the segment.

4. Claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over Rice *et al.* in view of Gifford as applied to claims 1-6, 8, and 18-24 above, and further in view of Nazarenko *et al.* (US 6090552).

The teachings of Rice *et al.* in view of Gifford are applied herein as applied in the previous rejection. Rice *et al.* in view of Gifford do not teach methods wherein the reference DNA is methylated at all CpG positions.

However, the inclusion of a methylated control in an assay for the determination of methylation would have been routine at the time the invention was made. Nazarenko *et al.* teach methods for detecting methylation in samples, and teach the inclusion of methylated control nucleic acids in these assays and in kits for performing these assays (see Col. 37-38 and 49-50, for example). Thus, It would have been *prima facie* obvious to one of ordinary skill in

the art at the time the invention was made to have included in the assays taught by Rice *et al.* in view of Gifford et al. a control which is methylated at all CpG positions as taught by Nazarenko *et al.* in order to have had a standard for comparison of all results against those obtained with a methylated control.

Response to Remarks

The remarks have been carefully considered but are not persuasive. All previously set forth rejections under 112 2nd paragraph are withdrawn in view of the amendment or cancellation of the rejected claims.

Applicant traverses the 103 rejections under Rice et al. in view of Gifford stating that the claimed method is very sensitive, appearing to attempt to assert an unexpected result of increased sensitivity. However, this is not persuasive. The argument is based upon the fact that within a given stretch of genomic DNA there may be more than one CpG position. There is no evidence on the record that the instant method is any more effective at detecting multiple methylated positions within a given sequence than the closest prior art at the time the invention was made. A showing of unexpected results must be made relative to the closest prior art (MPEP 716.02(e)). Rice et al. provide molecules that have more than one CpG position within them and detect these positions within a stretch of DNA. Since Rice et al. actually determine the methylation status of every possible position within the stretch of tested DNA it is not clear how the claimed invention can be construed as "more sensitive" than the closest prior art of Rice et al. Applicant has offered no evidence that their method accomplishes this task with an unexpected increased sensitivity. Thus, the arguments of record represent attorney arguments that are not supported by evidence on the record, and it is well settled that attorney arguments cannot take

the place of evidence (MPEP 716.01(c)). Further, applicant's argument to this end is not commensurate with the scope of the claims (see MPEP 716.02(d)). Applicant argues that in situations where a segment has multiple methylation positions, the claimed method will have enhanced sensitivity. However, the claim is sufficiently broad so as to encompass segments with a single methylation as well as many methylations.

Applicant's use of the word "sensitivity" appears to be misplaced. Sensitivity of an assay refers to the ability to detect the presence of very low copy number of a particular molecule or feature present in a sample, in this case, methylated sites. The closest prior art, in this case, Rice et al. provides a method in which the identity of every single position is determined after bisulfite analysis, and thus, every possible methylated position within a stretch of sample DNA would be detected. This would result in a highly sensitive method.

Applicant further argues on page 14 of the remarks that in Gifford, sensitivity is of lower importance. There is no evidence on the record to support the assertion that Gifford views sensitivity as "only of lower importance." In fact, as applicant points out, Gifford is interested in detecting rarely occurring molecules, which would require a highly sensitive method.

Applicant provides discussion about the limited amount of DNA present for methylation analysis. However, this problem is overcome by the teachings of Rice et al. who provide PCR amplification of test DNA, thus providing additional copies of DNA.

Applicant's arguments that Gifford allows the detection of only a SNP and not the identity of the change is a piecemeal analysis that does not consider the totality of the record, since the bisulfite treatment is provided in the methods taught by Rice et al. The comparison of bisulfite treated molecules to a control, as discussed in the rejection would allow the

determination of the methylation positions. In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).

Applicant further argues that the present invention "allows an easy quantification of methylation (p. 16-17 of the response)." In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., quantification of methylation) are not recited in the rejected claim(s). Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). In this case, the specification does not even appear to discuss methods wherein methylation is quantified. Again, however, it would seem that this comparison is not against the closest prior art of Rice et al. whose method clearly would allow quantification of methylated positions since it is determined whether or not each position in a segment is methylated. The unexpected property must be in comparison to the closest prior art.

Applicant argues that the present invention "allows simultaneous analysis of mosaic methylation (p. 17-18 of the remarks)." Again, this is a feature of the invention which is not claimed, nor is it discussed in the specification. Further still, this is not a property which is unexpected in view of the prior art, as the prior art teaches determining the methylation status of all positions within a segment of DNA.

The rejection is maintained.

Conclusion

5. No claim is allowed.

6. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Juliet C Switzer whose telephone number is (571) 272-0753. The examiner can normally be reached on Monday, Tuesday, or Thursday, from 9:00 AM until 4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached by calling (571) 272-0735.

The fax phone numbers for the organization where this application or proceeding is assigned are (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is

(571)272-0507.

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For all other customer support, please call the USPTO Call Center (UCC) at 800-786-9199.

Juliet C. Switzer
Primary Examiner
Art Unit 1634

November 13, 2006